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## STC-1 Cells

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# Chapter 19

## STC-1 Cells

**Triona McCarthy, Brian D. Green, Danielle Calderwood, Anna Gillespie, John F. Cryan, and Linda Giblin**

**Abstract** Gastrointestinal hormones such as cholecystokinin (CCK), glucagon like peptide 1 (GLP-1), and peptide YY (PYY) play an important role in suppressing hunger and controlling food intake. These satiety hormones are secreted from enteroendocrine cells present throughout the intestinal tract. The intestinal secretin tumor cell line (STC-1) possesses many features of native intestinal enteroendocrine cells. As such, STC-1 cells are routinely used in screening platforms to identify foods or compounds that modulate secretion of gastrointestinal hormones in vitro. This chapter describes this intestinal cell model focussing on it's applications, advantages and limitations. A general protocol is provided for challenging STC-1 cells with test compounds.

**Keywords** Enteroendocrine cells • Secretin tumor cell line (STC-1) • Satiety hormones • GLP-1 • CCK • PYY • Food components • G protein coupled receptors • Nutrient sensing

### 19.1 Origin

STC-1 was originally established from cells present in murine enteroendocrine tumours. These tumours arose in the duodenum of double transgenic mice expressing the rat insulin promoter linked to the simian virus 40 large T antigen and to the

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polyomavirus small T antigen (Rindi et al. 1990). STC-1 is a relatively slow growing cell line, with a doubling time of 54 h in standard growth media. They are not easily amenable to transfection with several studies reporting low transfection efficiencies (stable and transient). Under culturing conditions, this cell line is heterogenous. However most subpopulations demonstrate immunoreactivity to the gut hormone, cholecystokinin (CCK) (Rindi et al. 1990). As such, STC-1 cells were originally used as a model of native CCK-producing I-cells. Over the past two decades, the uses of the STC-1 cell line has been expanded to investigate (a) the cellular signaling mechanisms involved in secretion and gene transcription of other gut hormones (Cordier-Bussat et al. 1998; Geraedts et al. 2009), (b) enteroendocrine cell differentiation (Ratineau et al. 1997), (c) tumor cell growth (Bollard et al. 2013) and (d) intestinal immune responses (Palazzo et al. 2007).

## 19.2 Features and Mechanisms

In addition to CCK, STC-1 cells express and secrete a wide range of gut hormones known for their roles in metabolism, feeding and satiety, including glucose dependent insulinotropic polypeptide (GIP), peptide YY (PYY), pancreatic polypeptide, neurotensin and the proglucagon derived peptides [glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2) and oxyntomodulin] (Rindi et al. 1990; Hand et al. 2013). STC-1 cells secrete these hormones in response to a range of physiological stimuli, although levels may differ to native enteroendocrine cells (Reimann et al. 2008). This ability to secrete a variety of gut hormones ensures that the STC-1 cell line remains a popular ‘look-see’ choice with food researchers.

Monosaccharides (Mangel et al. 1994), fatty acids (Hand et al. 2010), aromatic amino acids (Cordier-Bussat 1998; Wang et al. 2011), peptidomimetic compounds (Geraedts et al. 2011) and bitter tastants (Miyata et al. 2014) have all been demonstrated to dose dependently elicit CCK and GLP-1 secretion from STC-1 cells. Several studies have also reported that sucralose and other sweeteners increase CCK and GLP-1 secretion in STC-1 cells (Geraedts et al. 2012). Interestingly, these substances do not appear to be GLP-1 stimulants in primary cultures of murine intestinal epithelium (Reimann et al. 2008). Although PYY secretion has been less widely investigated, STC-1 cells reliably produce and secrete PYY in response to a variety of fatty acids including valeric acid, linoleic acid and conjugated linoleic acid 9,11 (Hand et al. 2013). GIP expression and production by STC-1 cells is poor. Nevertheless as there are few in vitro alternatives, GIP subclones of STC-1 cells with increased *GIP* expression have been generated (Kieffer et al. 1995).

Several neural and hormonal stimuli which play an important role in coordinating early responses to food ingestion in vivo also induce gut peptide release from STC-1 cells. These include the neurotransmitters gamma-aminobutyric acid (GABA), acetylcholine, orexin, bombesin, apelin, gastrin releasing peptide as well as bile acids and leptin.

Hormone secretion from this cell line appears to be dependent on a rise in cytoplasmic cAMP and  $\text{Ca}^{2+}$  which ultimately leads to alteration in hormone gene transcription levels or hormone peptide release. Increased  $\text{Ca}^{2+}$  levels can occur as a result of (a) an influx of  $\text{Ca}^{2+}$  across the plasma membrane through activation of voltage-gated calcium channels or (b) inositol 1,4,5-triphosphate (IP3) triggered  $\text{Ca}^{2+}$  release from intracellular stores. In native enteroendocrine cells, intracellular levels of  $\text{Ca}^{2+}$  and cAMP are also altered by the activity of a number of G-protein coupled receptors (GPCR's) and nutrient transporters. STC-1 cells have been found to intrinsically express many of these important GPCRs including GPR40 and GPR120 (Tanaka et al. 2008). Hormone secretion is dependent on GPR40 in native murine I-cells, as linolenic acid induced CCK secretion was absent in cells lacking this receptor (Liou et al. 2011b). In contrast, GPR40 knockdown had little effect on the ability of STC-1 cells to sense fatty acids. Rather, it is GPR120 that appears to play a more important role in fatty acid sensing in STC-1 cells. Fatty acid-induced hormone release is impaired in *GPR120* siRNA silencing experiments (Tanaka et al. 2008). Wang *et al.* demonstrated that phenylalanine induced  $\text{Ca}^{2+}$  mobilization and hormone secretion occurs via the calcium-sensing receptor which is highly expressed in native murine enteroendocrine cells (Wang et al. 2011). This receptor is however poorly expressed in STC-1 cells, although it is functional. Several taste signalling elements such as alpha-gustducin, phospholipase C- $\beta$ 2, transient receptor potential channel M5 and the taste receptor T1R/T2R subunits are present and functional in STC-1 cells similar to native enteroendocrine cells (Dyer et al. 2005).

Recent evidence suggests that ATP-sensitive  $\text{K}^+$  channels and transporters [e.g. sodium-coupled glucose transporters (SGLTs) and peptide transporters (PEPTs)] contribute to hormone secretion in enteroendocrine cells (Tolhurst et al. 2009). However STC-1 cells express low levels of the  $\text{K}_{\text{ATP}}$  channel subunits, Kir6.2 and SUR1. Endogenous expression of PEPTs is also poor in STC-1 cells (Liou et al. 2011a).

In addition to stimulating gut hormone release, common luminal nutrients added to the growth media are also capable of modulating gene expression in STC-1 cells. As such this cell line has been widely used to investigate the regulation of *CCK* gene expression. However, for the *proglucagon* gene, aberrant posttranslational processing has been documented (Blache et al. 1994). In vivo, the synthesis of proglucagon-derived peptides is tissue specific due to the action of various prohormone convertase enzymes (e.g. PC1/3). In pancreatic  $\alpha$  cells, posttranslational processing of proglucagon liberates glucagon, glicentin-related pancreatic peptide, and the major proglucagon fragment, which contains the sequences for both GLP-1 and GLP-2. In contrast, processing of proglucagon in the intestinal enteroendocrine L-cell generates GLP-1 and GLP-2, as well as the glucagon-containing peptides, glicentin and oxyntomodulin. Blache *et al.* reported that proglucagon processing in STC-1 cells results in the production of significant quantities of glucagon in addition to glicentin and oxyntomodulin (Blache et al. 1994). Thus STC-1 cells appear to process the proglucagon fragment in a manner which is intermediate between intestinal L-cells and pancreatic  $\alpha$  cells.

### 19.3 Stability, Consistency and Reproducibility

When used appropriately, STC-1 cell line has been shown to be a reliable and reproducible enteroendocrine cell model. However, considerable variances in hormone secretion levels have been reported by different laboratories for the same substance (Geraedts et al. 2009; Hand et al. 2013). For example, Cordier-Bussat *et al.* showed that addition of protein hydrolysates from meat, casein and soybean to STC-1 cells increased CCK and GLP-1 release compared to undigested proteins (Cordier-Bussat et al. 1997). In contrast, other studies have demonstrated that intact protein is a much stronger stimulant for CCK and GLP-1 release in STC-1 cells than hydrolysates or specific peptides (Geraedts et al. 2011). In addition to differences between different laboratories, significant inter-experimental variability in the amount of CCK secreted has also been reported within study groups. Differences in culture protocols such as seeding density, cell feeding routine, washing steps, test buffer and passage number may account in part for this variability. However, inter-experimental variability is most likely a direct result of the heterogenous non-stable nature of the cell line. Cultured STC-1 cells demonstrate 95 % immunoreactivity to CCK, while only 7 % stain positively for GIP (Rindi et al. 1990). Glassmeier *et al.* reported that the sensitivity of STC-1 cells to the neurotransmitter GABA varied considerably from cell to cell, due to the heterogeneous density and expression of GABA<sub>A</sub> receptors in this cell line (Glassmeier et al. 1998). STC-1 cells may switch to multiple differentiated states during proliferation (Rindi et al. 1990). Consequently levels of hormone secretion may significantly differ from one test to another. To improve the homogeneity and the stability of STC-1 cells, several studies have attempted to isolate and characterize clonal cell lines from parental STC-1 cells. However even these cells do not express a stable phenotype. For example, while attempting to clone a pure GIP-expressing cell, Kieffer *et al.* observed that only 30 % of the expanded clone still expressed GIP immunoreactivity due to dedifferentiation of the cells (Kieffer et al. 1995). These observations highlight the importance of including adequate controls and replicates for each independent experiment. As early passage number STC-1 cells have greater heterogeneity, cells that have been passaged more than 10 times are recommended. It also underlines the importance of verifying results with experiments independent of STC-1 cells.

### 19.4 Relevance to Human In Vivo Situation

A major requirement of any in vitro model is that it should demonstrate a strong predictive power, yielding data that support interpretation of results for the in vivo situation. Although STC-1 cells do respond to a range of physiological stimuli shown to elicit gut hormone secretion in vivo, some inconsistencies have been observed. Hall *et al.* demonstrated that while whey consumption in humans resulted in a greater increase in GLP-1, GIP, and CCK plasma levels compared to other protein sources (Hall et al. 2003), whey protein hydrolysate are ineffective stimulants

of CCK release in STC-1 cells (Foltz et al. 2008). STC-1 and the native state also differ in PYY secretion to fatty acids (Hand et al. 2013).

In vivo gut hormone release occurs through direct sensing of luminal nutrients but also indirectly by vagal and humoral stimulation (Gribble 2012). Evidence shows that the release of bile acids, gastric acid secretions, mechanical stimulation by peristaltic contractions, gastric emptying and hormonal stimulation all result in gut hormone modulation. While STC-1 cells harbour many features of native intestinal hormone secreting cells, they are cultivated as monolayers on plastic surfaces and lack a normal cellular environment. They therefore cannot reproduce the integrative physiological interplay present in vivo. Thus many components which stimulate gut hormone secretion through indirect mechanisms will not be detected by this model. A typical example of this limitation is that potato protease inhibitors fail to elevate CCK expression or release in STC-1 cells (Komarnytsky et al. 2011). However in vivo potato protease inhibitors reduce food intake, and increase plasma and duodenal mucosal mRNA levels of *CCK* in rats indirectly by inhibiting trypsin mediated deactivation of endogenously produced CCK releasing factors (Komarnytsky et al. 2011).

It is also worth noting that compounds which elicit gut hormone secretion from STC-1 cells and in vivo, do not always effect food intake or the perception of the feeling of fullness. For example, Korean pine nut oil effectively increases CCK secretion from STC-1 cells and in plasma levels of humans. This increase in satiety hormone levels however did not correspond to a change in subjective appetite sensations as assessed by visual analogue scales (Pasman et al. 2008). This lack of relationship between increased plasma concentrations of gut hormones and appetite ratings has been observed in other studies (Veldhorst et al. 2009).

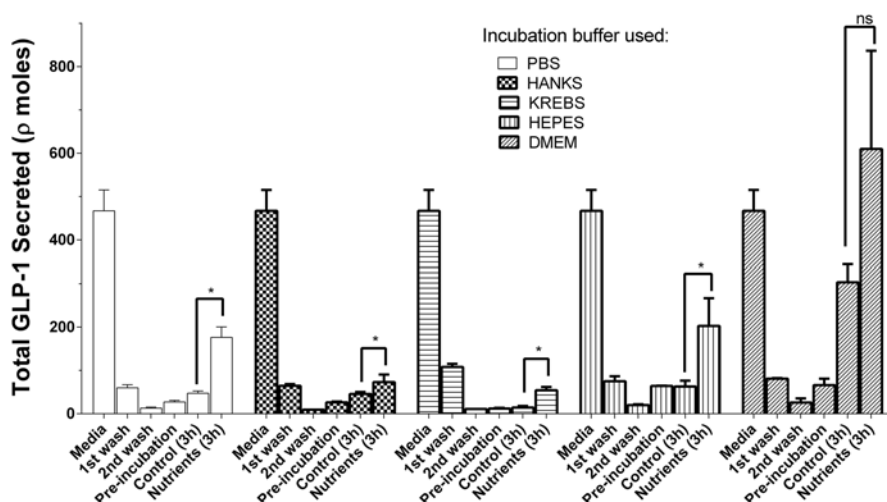
## 19.5 General Protocol

### 19.5.1 Cell Maintenance Protocol

STC-1 cells should be cultured in supplemented DMEM media (DMEM containing 4.5 g/l D-glucose, without sodium pyruvate) (GlutaMAX, GIBCO, Paisley, UK) with 17.5 % foetal bovine serum (FBS), 100 U/ml penicillin, 100 mg/l streptomycin and incubated in a 5 % CO<sub>2</sub> humidified atmosphere at 37 °C. Cells should be passaged at 80–90 % confluence. Passage number should be between 15 and 40.

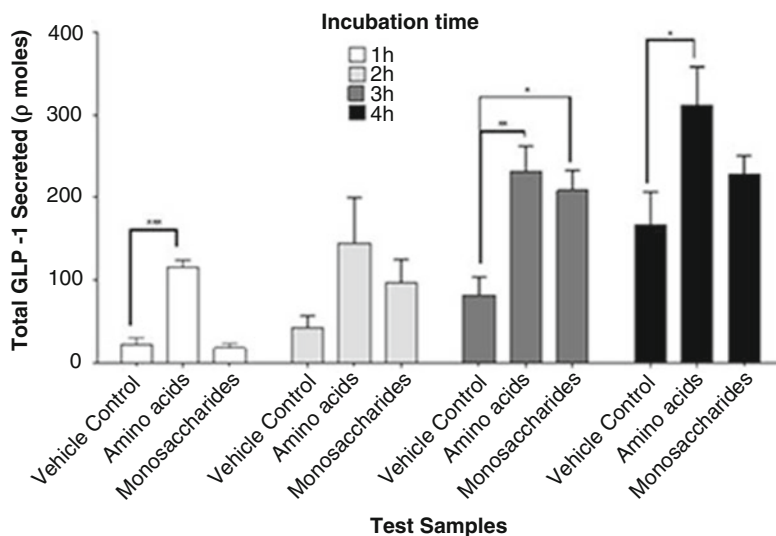
### 19.5.2 Experimental Protocol for Test Compounds

Test compounds should be prepared in pre-warmed buffer. Buffer should be tested to ensure compatibility with the particular immunoassay.



**Fig. 19.1** Effect of various incubation buffers on levels of Total GLP-1 secreted from STC-1 cells. STC-1 cells ( $2 \times 10^6$ ) were challenged with mixed nutrients [glutamine + valine + lysine + glycine + glucose + fructose (all at 40 mM)] for 3 h. Samples were prepared in PBS or HANKS or KREBS or HEPES buffers or DMEM media (GlutaMAX, GIBCO, Paisley, UK). The composition of the buffers are as follows; PBS (136.9 mM NaCl, 2.7 mM KCl, 10.1 mM  $\text{Na}_2\text{HPO}_4$ , 1.8 mM  $\text{KH}_2\text{PO}_4$ ), HANKS (136.9 mM NaCl, 5.4 mM KCl, 1.3 mM  $\text{CaCl}_2$ , 1 mM  $\text{MgSO}_4$ , 0.2 mM  $\text{NaH}_2\text{PO}_4$ , 0.4 mM  $\text{KH}_2\text{PO}_4$ , 4.2 mM  $\text{NaHCO}_3$ , 5.6 mM glucose), KREBS (118 mM NaCl, 4.7 mM KCl, 25 mM  $\text{NaHCO}_3$ , 1.25 mM  $\text{CaCl}_2$ , 1.2 mM  $\text{MgSO}_4$ , 1.2 mM  $\text{KH}_2\text{PO}_4$ , 11 mM glucose) and HEPES (140 mM NaCl, 4.5 mM KCl, 1.2 mM  $\text{CaCl}_2$ , 1.2 mM  $\text{MgCl}_2$ , 20 mM HEPES). Total GLP-1 was measured by a specific GLP-1 radioimmunoassay. Statistical comparisons (unpaired Student's t-test) were only performed on 3 h incubations with or without nutrient challenge for each selected buffer. Significant differences are indicated on the graph (\* $P < 0.05$ )

Figure 19.1 indicates that dramatically different results can be obtained depending on the buffer system used. Use of buffer solutions which are highly stimulatory (e.g. DMEM media which contains amino acids and vitamins), should be avoided because hormone levels for vehicle controls will be greatly elevated and can distort results. In previous experiments, we have favoured KREBS buffer. The test solution pH should be adjusted to 7.0–7.4 and filter sterilised (0.45  $\mu\text{m}$  filter). If solubility of test compounds is an issue (e.g. in the case of lipids or fatty acids) careful monitoring is needed to ensure test compounds remain in solution for the duration of experiments. To measure acute hormone secretion, STC-1 cells should be seeded into 6 well plates at  $1.5 \times 10^6$  cells in supplemented DMEM media. After 18 h at 37 °C in 5 %  $\text{CO}_2$ , media should be aspirated and cell monolayers washed 1–2 times with selected buffer. The cells should be acclimatised in buffer for 1 h. Culture media and wash buffers can be kept to ensure that cells have reached basal levels of hormone secretion (Fig. 19.1). After 1 h, the buffer is aspirated and 1 ml test solution added to wells. Cells are then incubated for 1–4 h at 37 °C, 5 %  $\text{CO}_2$  (in our experience a period of 3–4 h is optimal for hormone secretion, Fig. 19.2). Positive controls known to stimulate gut hormone secretion and negative controls (buffer alone)



**Fig. 19.2** Determining the optimal incubation time to measure levels of Total GLP-1 secreted from STC-1 cells. STC-1 cells ( $2 \times 10^6$ ) were exposed for various incubation times with stimulatory amino acid solution (40 mM glutamine, 40 mM valine, 40 mM lysine and 40 mM glycine) and monosaccharide solution (40 mM glucose and 40 mM fructose) which were prepared in HEPES buffer. Data were statistically compared using the unpaired Student's t-test and significant differences to vehicle controls are indicated \*P<0.05, \*\*P<0.01 and \*\*\*P<0.001

should always be included in each experimental unit. Post-incubation, we recommend the addition of 10  $\mu$ l 10X Halt Protease and Phosphatase Inhibitor (Thermo Fisher Scientific, USA) to protect against gut hormone degradation. Cellular supernatants are collected and store at  $-80^\circ\text{C}$  prior to further analysis. It is best practice to immediately transfer solutions to tubes placed on ice and to centrifugate ( $900 \times g$  for 5 min) to remove cellular debris. At least three biological replicates should be performed.

## 19.6 Assess Viability

Prior to hormone studies, viability or integrity of STC-1 cells challenged with test compounds at various concentrations should be tested. Where test compounds are digested, viability tests should be performed with the inactivated and pH adjusted (7.0–7.4) digestion matrix. An array of methods and kit-based assays are available for cell viability (e.g. measurement of mitochondrial reductase with tetrazolium salts, measurement of cell membrane integrity or signs of cytolysis and/or detection of increased apoptosis). Due the ability of healthy STC-1 cells to change morphology and grow extensions, systems based on electrical impedance may be unsuitable for such tests.



## 19.7 Experimental Read out

Intracellular levels of the secondary messengers (cAMP, IP3 and  $\text{Ca}^{2+}$ ) are often used as rapid indirect indicators of gut hormone secretion. Several types of assays are currently available to measure cAMP including radioimmunoassay approaches, time-resolved FRET-bioluminescence resonance energy transfer based assays, enzyme fragmentation complementation and cyclic nucleotide gated ion channel coupled assays. Intracellular  $\text{Ca}^{2+}$  concentrations can be determined directly using membrane permanent calcium-sensitive dyes (e.g. Fluo-3, Fluo-4 or Fura-2) that fluoresce upon exposure to free  $\text{Ca}^{2+}$ . IP1, a downstream metabolite of IP3, can be used as a surrogate for direct determination of  $\text{Ca}^{2+}$  as it has a longer half-life. A competitive immunoassay is commercially available that uses terium cryptate-labeled anti-IP1 and D2 labeled IP1 (Cisbo Bioassays, France). IP1 levels only reflect release of  $\text{Ca}^{2+}$  from intracellular stores and do not account for increased  $\text{Ca}^{2+}$  levels resulting from influx across the cellular membrane.

Specific assays for individual hormones include qRT-PCR assays and immunoassays (ELISA and RIA). For qRT-PCR, standard methods apply. Result interpretation is important as increases in mRNA levels and intracellular peptide levels may not result in corresponding increases in secretion levels of gut peptides (Hand et al. 2010). A number of sensitive and accurate commercial immunoassays (ELISA and RIA) are available for the detection of CCK, PYY and GLP-1, although multiplexing these analytes has proved difficult. Antibodies may not discriminate between bioactive and inactive forms of gut peptides. Moreover, a large cross-reactivity with gastrin has been observed for many commercially available assays. To overcome these limitations Foltz *et al.* developed an assay based on the ability of dietary components to induce CCK release from STC-1 cells and in turn activate the CCK receptor overexpressed in the chinese hamster ovary cell line (Foltz et al. 2008). Upon activation of the CCK receptor,  $\text{Ca}^{2+}$  levels increase, which can be visualized with a fluorescent dye. Thus this assay rapidly and directly determines the effect of a nutritional intervention on activation of CCK receptor yielding results which are more physiological relevant.

## 19.8 Conclusions

The murine STC-1 cell line is regarded as ‘best in class’ to evaluate secretion of several satiety hormones in response to food components *in vitro*. The gut hormones CCK, GLP-1, GIP, PYY, pancreatic polypeptide, neurotensin, GLP-2 and oxyntomodulin are all secreted by STC-1 cells. However the heterogeneous nature of STC-1 cells can introduce discrepancies from experiment to experiment. In addition, its response to stimulants can vary from the native enteroendocrine cell which underlines the importance of substantiating results *in vivo*.

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